Human Epidermal Keratinocytes for iPS Cell reprogramming

Complete Kit (Cat.# PC503hEKTN-K)
Cells Only (Cat.# PC503hEKTN-C)

User Manual

Store kit at -80°C on receipt

ver. 121009-001

Contents

List of Components

The Human Epidermal Keratinocytes, neonatal (HEKn), are available as either complete culture kit (Cat# PC503hEKTN-K) or cryopreserved cells (Cat# PC503hEKTN-C) in one vial.

HEKn, complete kit		Cat# PC503hEKTN-K
Component	Quantity	Ship
HEKn cells	$1x10^6$ cells/vial	Cryopreserved and shipped on dry ice
HEKn, supplemented culture medium	250 ml	Store at 4^o C upon receipt
Trypsin neutralization solution	50 ml TNS	Store at 4^o C upon receipt
HEKn, cryopreserved		Cat# PC503hEKTN-C
HEKn cells	$1x10^6$ cells/vial	Cryopreserved and shipped on dry ice

The cryopreserved HEKn cells are shipped on dry ice and should be immediately stored in liquid nitrogen upon receipt. Properly stored cells are stable for more than 2 years from the date received.

I. Human Epidermal Keratinocytes, neonatal

A. Description

Human epidermal keratinocyte, neonatal (HEKn) were isolated from individual neonatal foreskin (Day 1 to Day 3 of age). Each vial of this product contains $1X10^6$ viable cells. In our laboratory, each lot of cells is performance tested by culturing the cells through multiple passages in the absence of antibiotics and antimycotics, no contamination was observed during this culture period. Upon thawing, the cells are guaranteed to be >75% viable and have a potential of >30 population doublings when cultured according to the instructions provided in this manual.

B. Maintenance of HEKn Cultures

Culture medium
We recommend serum-free medium supplemented with keratinocyte growth supplement. The supplemented medium included in the HEKn complete kit is complete culture medium optimized for HEKn. HEKn culture medium can also be purchased from either Invitrogen(Medium 154CF, Cat# M-154CF-500) or ATCC (Cat# PCS-200-030) and must be supplemented with calcium plus their respective keratincoyte Growth Supplement (Invitrogen: HKGS, Cat# S-001-5; ATCC Cat# PCS-200-040). We recommend a low calcium concentration (0.05 ~ 0.07 mM) to slow HEKn differentiation, the calcium concentration in the supplement medium in our kit is 0.07 mM.

Trypsion/EDTA: 0.25% trypsin-EDTA

Trypsin neutralization solution (TNS): 5% Chelexed Fetal Bovine Serum in Hanks BSS

Initiating Cultures from Cryopreserved Cells

To insure the highest level of viability, be sure to warm medium to 37°C before using it on the cells. We recommend seeding cells recovered from cryoperservation at a cell density of 4×10^3 viable cells/cm^2 or higher. The procedure given below is a sample protocol for establishing cultures from the contents of one vial.

1. Prepare a bottle of supplemented keratinocyte growth medium according to the instructions supplied with that product.
2. Remove the vial from liquid nitrogen storage, taking care to protect hands and eyes.
3. Dip the lower half of the vial into a 37^0 C water bath to thaw.
4. When the contents of the vial have thawed, wipe the outside of the vial with 75% alcohol to disinfect and move the vial to a laminar flow culture hood.
5. Open the vial and pipet the cell suspension up and transfer the cells into a 15-cm conical tube with 10 ml fresh keratinocyte growth medium.
6. Pipet up and down with a 10 ml pipette to disperse the cells and centrifuge the cells at 180 g for 5 minutes. Observe the cell pellet.
7. Remove the supernatant form the tube, being careful not to dislodge the cell pellet.
8. Dilute the cells with fresh culture medium and seed new culture vessels with 4×10^3 cells/cm^2.
9. Incubate the cultures in a 37°C, 5% CO_2 /95% air humidified cell culture incubator.
10. Do not disturb the culture for at least 24 hours after the culture has been initiated.

Maintaining Stock Cultures

1. Change the culture medium 24 to 36 hours after establishing the secondary culture from cryopreserved cells.
2. Change the medium every other day thereafter, until the culture is approximately 80% confluent.

Note: Do not allow stock culture to exceed 80% confluent to prevent differentiation of HEKn.

Subculturing HEKn

View the culture under the microscope to confirm that the cells are subconfluent before splitting. The following protocol is designed for the subculture of one 75 cm^2 culture flask.

1. Remove all of the culture medium from the flask.
2. Add 3 ml of 0.25% trypsin-EDTA solution to the flask. Rock the flask to ensure that the entire surfaced is covered.
3. Incubate the flask at room temperature until the cells have become completely round, approximately 5-10 minutes. View the culture frequently under a microscope to avoid over digestion.
4. Add 7 ml of trypsin neutralization solution to the flask and transfer the detached cells to a sterile 15 ml conical tube.
5. Centrifuge the cells at 180 g for 5 minutes.
6. Remove the supernatant from the tube, being careful not to dislodge the cell pellet.
7. Resuspend the cells pellet in 10 ml supplemented medium. Pipet the cells up and down with a 10 ml pipette to ensure a homogeneous cell suspension.
8. Determine the concentration of cells in the suspension.
9. Seed new culture vessels with 4×10^3 cells/cm^2, or 1 to 4 split if starting cells at a 80% confluence.
10. Incubate the cultures in a 37 °C, 5% CO_2 /95% air humidified cell culture incubator.

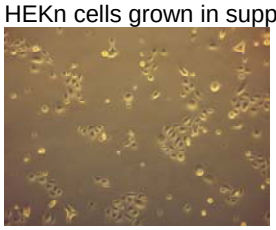
Cryopreserving HEKn

1. Follow steps 1-6 from the Subculturing of Cells above.
2. Resuspend the cell pellet in supplemented medium. Add approximately 1 ml for each T75 flask.
3. Count the number of cells and dilute the cell suspension to 2 x 10^6 cells/ml.
4. Add an equal volume of cold 2X Freezing Media to the cell suspension.
5. Aliquot 1 ml of suspension into each cryovial (1 x 10^6 cells/vial).
6. Place the vials in a cell-freezing container and keep it at -80 °C overnight.
7. Transfer the vials to a liquid nitrogen tank for long-term storage.

C. Product Information

Organism: *Homo sapiens* (human)
Gender: Male
Age: Neonatal (Day 1 to Day 3)
Morphology: Cobblestone appearance
Comments: The serum-free media is formulated to inhibit fibroblast growth and the low calcium concentration 50~70 µM slows differentiation. No feeder layers or extracellular matrix proteins are required.

HEKn cells grown in supplemented keratinocyte growth medium. Images of human epidermal keratincoytes in culture after thaw.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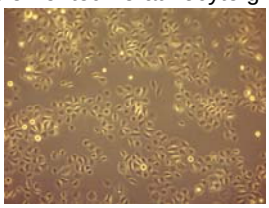
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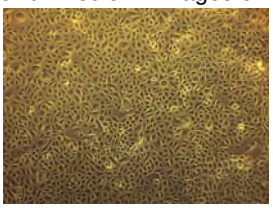
Day 6 -HEKn

D. References

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Carey BW et al. 2009. Reprogramming of murine and human somatic cells using a single polycistronic vector. Proc Natl Acad Sci. 106:157-62.

Takahashi, K. and Yamanaka, S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663–676.

Takahashi K. et al. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 131: 861–72.

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E. Related Products

- **Human Foreskin Fibroblasts, neonatal as reprogramming source cell (Cat. # PC501A-HFF)**
  From mesoderm origin, these neonatal HFFn are isolated from individual neonatal foreskin and therefore have a single genetic background. High purity and low passage of these neonatal HFFn (Day 1 to Day 3 of age) provides a reliable cell source for efficient reprogramming.

- **Human Foreskin Fibroblasts, neonatal as feeder cells (Cat. # PC502B-HFF)**.
  These pooled HFFs are optimized to provide a balanced nutrition to support your ES cell culture and reprogramming experiment. High purity and low passage of neonatal HFFn (Day 1 to Day 3 of age) are characteristics of this product.

F. Technical Support

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http://www.systembio.com

For additional information or technical assistance, please call or email us at:

System Biosciences (SBI)
1616 North Shoreline Blvd.
Mountain View, CA 94043

Phone: (650) 968-2200
(888) 266-5066 (Toll Free)

Fax: (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

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